UNITED STATES DEPARTMENT OF AGRICULTURE
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THE PHOTOMETRIC DETERMINATION OF PROTEIN IN WHEAT

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The importance of protein content as an index of the processing value of wheat is well recognized throughout the grain trade. Protein often is a major factor influencing the market value of wheat, particularly of the classes Hard Red Winter and Hard Red Spring. Although the texture determination used in the inspection of these classes of wheat under the Official Grain Standards of the United States correlates in a rough way with protein content, and in some instances appears to be an even more useful index of quality than protein, the Department has been criticized frequently for not utilizing the protein test in the inspection of wheat.

One of the practical reasons for not incorporating protein as a factor in the standards for wheat is that the method and the equipment necessary for making protein tests in volume are too cumbersome and expensive to be readily available at the numerous points where inspections are made. The Agricultural Marketing Service, therefore, is interested in the development of a more simple, rapid, and serviceable method for making protein tests.

A survey of the literature on the subject indicated that in the field of cereal chemistry as well as of other branches of plant chemistry, practically all efforts to simplify the protein test have been confined to the development of minor modifications of the well-known Kjeldahl procedure. In the field of clinical medicine, on the other hand, numerous simple and rapid methods have been developed in recent years for the determination of protein in body fluids such as blood, cerebro-spinal fluid, and urine. These methods, for the most part, have been based on the measurement of color intensity by the photoelectric cell or so-called "electric eye."

Preliminary attempts to adapt some of these clinical methods to the determination of protein in wheat and flour showed some promise but in the course of these investigations a much more promising method based on the peculiar physico-chemical properties of wheat gluten was devised. This method is entirely different in principle from the Kjeldahl procedure now used for wheat protein determinations, and it resembles the above-mentioned clinical methods only insofar as the final measurement is made with a photometer using the photoelectric cell.

The method is based on the principle that the proteins of wheat are readily dissolved by shaking the fine wheat meal with a very dilute solution of alkali. By neutralizing the alkali in a portion of such a protein solution with a suitable "buffer solution" minute particles of the gluten protein hardly visible with a microscope are formed and remain suspended in the solution giving it a turbid or cloudy appearance. The degree of turbidity developed is an index of the gluten protein content of the wheat used in the test and may be determined quickly and accurately with the photoelectric photometer. In addition to the relative simplicity of the photometric method it has other important advantages over the conventional protein test for wheat.

The Kjeldahl test has two fundamental weaknesses when applied to the determination of wheat protein. In the first place it is in reality a test for nitrogen rather than for protein content, the protein calculation being based on the assumption that all the nitrogen present in the wheat is in the form of protein. Thus for a sample of wheat in which the protein has become partially destroyed by the action of insects or by improper storage, the Kjeldahl test would measure all protein decomposition products as protein. Such a sample would test the same as a sound sample with the same nitrogen content, although its true protein content might be substantially less. The photometric test, on the other hand, does not react to most protein decomposition products.

In the second place, the Kjeldahl test does not differentiate in any way between the different types of protein present in the wheat kernel. A considerable part of the wheat protein consists of the nonglutenous proteins of the bran and germ which are of little or no importance from the standpoint of ultimate flour quality. The percentage of these nonglutenous proteins is quite variable and depends in considerable measure on the degree of plumpness of the kernels. Thus the abnormally high protein values often found in shriveled wheats are in many instances largely the result of a high content of nongluten protein. The photometric protein test determines essentially only the gluten proteins of the inner part of the wheat kernel (endosperm) from which flour is made and should, therefore, provide a better index of ultimate flour quality than does the total protein value obtained by the conventional method.

The photometric procedure is carried out in the following manner:

1. The wheat is ground to a fine meal similar to that used for the standard Kjeldahl procedure.

2. A carefully weighed 0.5-gram portion of this meal is shaken for 2 or 3 minutes with a 100 ml. portion of a very dilute solution of alkali (0.05 N. KOH), to extract the protein.

3. The mixture is centrifuged for 10 minutes at about 1,800 r.p.m. to separate the starch and bran particles from the protein solution.

4. To a 5-ml. portion of the protein solution is added 25 ml. of a special phosphate buffer solution to produce the colloidal suspension of gluten protein.

5. After the suspension has stood for an hour, its light transmission is determined with the photoelectric photometer, and the gluten protein content of the wheat is determined from a previously prepared calibration chart, or the instrument may be calibrated so that it can be read directly in terms of gluten protein content.

It should be distinctly understood that this photometric method is still in the process of development. Certain difficulties, chiefly mechanical difficulties with the equipment and its standardization, prevent us from recommending the adoption of the method in commercial work at this time. We are confident, however, that the research we are now conducting and the collaborative assistance that has been promised us will eliminate these difficulties and that the method will develop into a valuable tool for use in the commercial evaluation of wheat.